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INFLUENCE OF CHEMICAL FORM, FEEDING REGIMEN, AND ANIMAL SPECIES ON THE GASTROINTESTINAL ABSORPTION OF PLUTONIUM

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ABSTRACT

We evaluated the effect of chemical form and feeding regimen on the gastrointestinal (GI) absorption of plutonium in adult mice at plutonium concentrations relevant to the establishment of drinking water standards. Mean fractional GI absorption values in fasted adult mice were: Pu(VI) bicarbonate, 15 x 10^{-4}; Pu(IV) bicarbonate, 20 x 10^{-4}; Pu(IV) nitrate (pH2), 17 x 10^{-4}; Pu(IV) citrate, 24 x 10^{-4}; and Pu(IV) polymer, 3 x 10^{-4}. Values in fed adult mice were: Pu(VI) bicarbonate, 1.4 x 10^{-4}; Pu(IV) polymer, 0.3 x 10^{-4}. Pu(VI) is the oxidation state in chlorinated drinking waters and Pu(IV) is the oxidation state in many untreated natural waters.

To assess the validity of extrapolating data from mice to humans, we also determined the GI absorption of Pu(VI) bicarbonate in adult baboons with a dual-isotope method that does not require animal sacrifice. Fractional GI absorption values obtained by this method were 23 ± 10 x 10^{-4} for fasted baboons (n=5) and 1.4 ± 0.9 x 10^{-4} for fed baboons (n=3). We have so far validated this method in one baboon and are currently completing validation in two additional animals.

At low plutonium concentrations, plutonium oxidation state [Pu(VI) vs. Pu(IV)] and administration medium (bicarbonate vs. nitrate vs. citrate) had little effect on the GI absorption of plutonium in mice. Formation of Pu(IV) polymers and animal feeding decreased the GI absorption of plutonium 5- to 10-fold. The GI absorption of Pu(VI) bicarbonate in both fed and fasted adult baboons appeared to be the same as in fed and fasted adult mice, respectively.

1. INTRODUCTION

We are studying the gastrointestinal (GI) absorption of plutonium under conditions relevant to establishing drinking water standards. The primary oxidation state we have studied is Pu(VI), the state present in chlorinated drinking water.[1] The medium is dilute bicarbonate, the major anion in Lake Michigan and many other drinking waters. Plutonium concentrations in administered solutions are low, 1 x 10^{-10} M, the molar concentration of ^{239}Pu at the maximum permissible concentration for plutonium in drinking water (5 pCi/ml; 0.18 Bq/ml).[2]

The International Commission on Radiological Protection (ICRP) is evaluating data to recommend a GI absorption value for use in establishing standards for oral exposure to plutonium in an environmental setting.[3] Previous ICRP recommendations have pertained to occupational exposures only.[4] Two areas that need to be considered in evaluating GI absorption data for establishing standards are (1) the effect of chemical form on the GI absorption of plutonium and (2) the extrapolation of data obtained in laboratory animals to humans. These two areas are addressed in the study reported here.

Our results compare the GI absorption of Pu(VI) in bicarbonate medium with that of Pu(IV) in various administration media used by other investigators.[5-10] We also report results of a study in progress on the GI absorption of Pu(VI) bicarbonate in both fed and fasted adult baboons. These results will provide insight into extrapolation of rodent data to humans.

2. MATERIALS AND METHODS

2.1 Plutonium Solutions

Four types of solutions of plutonium were prepared as described in detail elsewhere.[11] These solutions were approximately 5 x 10^{-10} M in plutonium and had the following compositions:

(1) Pu(VI) in 0.01 M $NaHCO_3$, 1 ppm chlorine, pH 8.3

(2) Pu(IV) in 0.01 M $NaHCO_3$, 0.01 M NaI, pH 8.3

(3) Pu(IV) in 0.01 M HNO_3, pH 2

(4) Pu(IV) in 0.17 M citrate buffer, pH 4.5

The plutonium isotope was $^{237}/^{239}$Pu, ^{236}Pu, or ^{238}Pu. The amounts of plutonium in the administered solutions, as well as the percentages in the IV and VI states, were determined by the lanthanum fluoride method.[12] The percentage of ultrafilterable plutonium (% UF) was determined by the procedure of Lindenbaum and Westfall.[13] This test gives a measure of the degree of polymerization of Pu(IV) in solution. A high % UF value indicates a low percentage of polymeric plutonium.

2.2 Plutonium Determinations

Blood, urine, and soft tissues were digested in concentrated HNO_3 prior to analysis for ^{236}Pu or ^{238}Pu. Bone samples were ashed in a muffle furnace at 600°C, dissolved in concentrated HNO_3, and diluted to a known volume

the dual-isotope method described by De~azia et al.[15] for determining the GI absorption of calcium in humans. Fo. each simultaneous administration of ^{236}Pu orally and ^{238}Pu intravenously, four to six values of GI absorption were calculated from (1) the oral/intravenous(iv) activity ratio in individual samples of blood, urine, liver, and caudal vertebrae and (2) the amounts of each isotope administered:

$$\text{fractional GI absorption} = \frac{S236}{S238} \times \frac{A238}{A236}$$

where S236 and S238 are, respectively, the amounts of ^{236}Pu and ^{238}Pu in a sample S of blood, urine, liver, or caudal vertebrae and A238 and A236 are, respectively, the amounts of ^{238}Pu administered intravenously and ^{236}Pu administered orally. For the ^{239}Pu administrations on Day 0, two to three values of GI absorption were calculated from the oral/iv activity ratios ($^{239}Pu/^{238}Pu$) in samples of liver and caudal vertebrae. We validated the dual-isotope method for Baboon B1 by comparing the GI absorption value calculated for the fasted baboon with that directly determined from analysis of the orally administered isotope excreted in urine and that retained by the whole baboon at sacrifice. Further validation of the method by direct analysis of the remaining two sacrificed baboons is in progress.

3. RESULTS

3.1 Effect of Chemical Form of Plutonium

The fractional GI absorption of plutonium in fasted mice was approximately 2×10^{-3} (0.2%) independent of the plutonium oxidation state, Pu(VI) vs. Pu(IV) (Groups A vs. B, Table I) and of the administration medium for Pu(IV), bicarbonate vs. nitric acid vs. citrate (Groups B vs. C vs. D). In contrast, the absorption of polymerized Pu(IV) was five- to sevenfold less than unpolymerized plutonium in both fasted (Groups B vs. E) and fed (Groups F vs. G) mice. The fractional GI absorption of Pu(VI) in fasted mice, 1.5×10^{-3} (Group A), was 10-fold greater than in fed mice, 1.4×10^{-4} (Group F).

3.2 Effect of Animal Species: Mouse vs. Baboon

Individual values for the fractional GI absorption of Pu(VI) in fasted, adult baboons are shown in Table II. The mean value for all animals was 2.3×10^{-3} (0.23%), almost the same as that in fasted mice (Table I, Group A). The mean value of Pu(VI) in fed adult baboons was 1.4×10^{-4} (0.014%), 16-fold lower than in fasted animals. Again the value in fed baboons is essentially the same as that in fed mice (Table I, Group F).

As shown in Table II (fasted animals, last line), the fractional GI absorption of Pu(VI) in one overnight-fasted "baboon without breakfast" was 2.8×10^{-3} (0.28%), 20-fold greater than in fed baboons and similar to that in 24-h fasted baboons.

For Baboon B1, the fractional GI absorption value directly determined for the fasted animal by analysis of ^{236}Pu excreted in urine and that retained in the baboon at sacrifice was 2.1×10^{-4}. This value is nearly identical to the mean value, 2.3×10^{-4}, calculated with the dual-isotope method (Table II).

TABLE I Influence of chemical form on the gastrointestinal absorption of plutonium in fed and fasted mice.

Experiment	Group Designation	Feeding Regimen	Oral Plutonium Solution					Fraction Retained ($x10^4$)[a]	
			% UF	Oxidation State VI	Oxidation State IV	Medium	n	Total	Liver
Pu(VI)	A	Fasted	85	12	88	0.01 M $NaHCO_3$	20	15 ± 3	3 ± 1
Pu(IV), high % UF	B	Fasted	70	100	0	0.01 M $NaHCO_3$	21	20 ± 2	3 ± 1
Pu(IV), nitric acid	C	Fasted	53	100	0	0.01 M HNO_3	12	17 ± 3	4 ± 1
Pu(IV), citrate buffer	D	Fasted	84	100	0	0.17 M citrate	8	24 ± 5	5 ± 1
Pu(IV), low % UF	E	Fasted	10	97	3	0.01 M $NaHCO_3$	3	3 ± 1	0.9 ± 0.3
Pu(VI)	F	Fed	99	0	100	0.01 M $NaHCO_3$	12	1.4 ± 0.2	0.4 ± 0.1
Pu(IV), low % UF	G	Fed	25	99	1	0.01 M $NaHCO_3$	8	0.31 ± 0.02	0.10 ± 0.01

[a]Values are means ± SE for the number of mice shown, n. Mice were sacrificed 6 days after oral plutonium administration.

4. DISCUSSION

4.1 Effect of Chemical Form of Plutonium

Studies of the behavior of plutonium in fresh water systems have shown that fallout plutonium in solution (that not bound to sediments) is in the form of Pu(V) or of Pu(IV) complexed to humic acids.[16,17] It has also been shown that the chlorination of drinking water results in the oxidation of soluble plutonium to Pu(VI).[1] Our studies have focused on the GI absorption of Pu(VI) because most drinking waters consumed by large populations are chlorinated. We have shown that the fractional GI absorption of Pu(VI) in fasted mice is 15×10^{-4} and in fed mice is 10-fold lower, 1.4×10^{-4} (Table I). These values need to be considered in deriving a standard for exposure to plutonium in drinking water.

To be relevant to the establishment of drinking water standards, studies of plutonium GI absorption must, as a first approach, involve administration of chemical forms of plutonium present in drinking water. However, we need to know if results of studies of the GI absorption of plutonium in chemical forms other than those in drinking water, e.g., Pu(IV) in nitric acid or in citrate buffer, can be used to set drinking water standards. Our results demonstrate that the GI absorption of Pu(IV) is the same as that of Pu(VI) if the administered Pu(IV) is not polymerized (Table I). Under these same conditions, the GI absorption of Pu(IV) is also independent of administration medium: 0.01 M bicarbonate, 0.01 M nitric acid, or 0.17 M citrate buffer (Table I). These conclusions could not have been reached from our data, however, had we not

TABLE II Gastrointestinal absorption of plutonium in fed and fasted adult baboons.

Animal Number	Oral Plutonium Solution: Isotope	Oxidation State IV	Oxidation State VI	% UF	Fractional GI Absorption ($\times 10^4$)
FASTED ANIMALS[a]					
B1	^{236}Pu	1	99	100	2.2 ± 0.2 (4)[b]
B2	^{236}Pu	20	80	44	11 ± 2 (6)[b]
B3	^{236}Pu	8	92	83	62 ± 10 (5)[b]
B4	^{236}Pu	32	68	74	14 ± 3 (5)[b]
B2	^{239}Pu	7	93	95	28 ± 10 (3)[b]
				Mean ± SE (n)	23 ± 10 (5)[c]
FED ANIMALS					
B1	^{239}Pu	19	81	100	3.3 ± 1.5 (2)[b]
B3	^{239}Pu	66	34	83	0.49 ± 0.03 (2)[b]
B4	^{239}Pu	27	73	97	0.47 ± 0.05 (3)[b]
				Mean ± SE (n)	1.4 ± 0.9 (3)[c]

[a]Baboons were fasted for 24 h prior to the ^{236}Pu administrations. Baboon B2 was fasted for 14 h prior to the ^{239}Pu administration to simulate a "baboon without breakfast."

[b]Value presented is the mean ± SE for the number of calculated GI absorption values, n, shown in parentheses. Calculated values were obtained as described in the text.

[c]Summary value for all fasted or all fed animals is the mean ± SE for the number of animals, n, shown in parentheses.

characterized our solutions of Pu(IV) with respect to polymer formation: the GI absorption of Pu(IV) in fasted mice with one solution was seven-fold greater than with a second solution, the latter being the one containing polymers (10% ultrafilterable, Table I). Analogous results were obtained with fed mice, again because we were not able to reproducibly prepare solutions of Pu(IV) in 0.01 M $NaHCO_3$ medium that were not polymerized. This problem does not arise with solutions of Pu(VI), because this form of plutonium is not subject to polymerization, regardless of the method of preparation or solution conditions.

The mean fractional GI absorption values in fed adult rats reported in the literature for Pu(IV) in 0.01 M nitric acid show an approximate 10-fold range, from a low of 3×10^{-5} obtained in experiments of Weeks et al.[6] to a high of 48×10^{-5} reported by Sullivan et al.[8] This 10-fold range is what might be expected if the lowest GI absorption value were obtained with solutions of Pu(IV) that contained polymers and the highest value with solutions that did not. Support for this explanation comes

from the observation that our value for Pu(IV) absorption in fed adult mice when the Pu(IV) was polymerized, 3×10^{-5} (25% UF solution, Table I), is the same as the low value for fed adult rats reported by Weeks et al.[6] The similarity of these values indicates that the Pu(IV) in the 0.01 M HNO_3 solutions of Weeks et al. may have been polymerized to an extent similar to that for the plutonium in our 25% UF solution. In addition, the value obtained by Sullivan et al.[8] for the GI absorption of Pu(VI) is 28×10^{-5}, similar to the high GI absorption value for Pu(IV), 48×10^{-5}.

The possibility also exists that Pu(IV) solutions prepared in citrate buffer, where a complexing anion is present to prevent polymerization, can contain polymers. GI absorption values reported by Weeks et al.[6] for Pu(IV) in citrate buffer also show a 10-fold range, from 3×10^{-4} to 4×10^{-3}.

We conclude from the above observations that the GI absorption values reported in the literature determined for Pu(IV) rather than Pu(VI) must be from studies in which the Pu(IV) solutions were characterized and shown to be low in polymers if they are to be used as primary data for the establishment of drinking water standards. Because most investigators do not analyze their Pu(IV) solutions for polymers, the most reliable GI absorption values for the establishment of drinking water standards come from studies of Pu(VI) absorption, the form of plutonium in chlorinated drinking waters. Information is still needed on the GI absorption of other forms of plutonium in natural waters, e.g., Pu(IV) complexed to humic acids. We are currently conducting studies in this area.

4.2 Effect of Animal Species: Mouse vs. Baboon

We became interested in comparing the GI absorption of plutonium in mice vs. baboons when we observed that the GI absorption values of two other metals, cadmium and lead, were 10-fold lower in our ANL mice than in humans and that GI absorption values for these metals in baboons (for lead) and monkeys (for cadmium) were the same as in humans.[11] Early results reported here from an experiment in progress demonstrate that, unlike those of cadmium and lead, the GI absorption values of Pu(VI) in fed and fasted baboons appear to be the same as those in our fed and fasted ANL mice, respectively. These results, once finalized, will allow us to extrapolate the GI absorption data that we and others have obtained in mice and rats to humans with greater confidence.

Good agreement between the GI absorption value of ^{236}Pu in fasted baboon B1 determined by the dual isotope method, 2.2×10^{-4}, and the value determined by analysis of tissues and excreta, 2.1×10^{-4}, validates in one baboon the dual isotope method for determining the GI absorption of plutonium. Baboons B2 and B4 have also been sacrificed, and samples of tissues and urine are currently being analyzed to provide a GI absorption value by direct assay to be compared with the calculated values reported here. If results are positive, we will have validated a useful non-sacrifice method for determining plutonium GI absorption values based on assay of blood and urine samples only, allowing multiple measurements of GI absorption to be made sequentially in the same animal.

5. CONCLUSIONS

Our results on the GI absorption of Pu(VI) in mice and baboons indicate that GI absorption values that need to be considered in setting drinking water standards are 2×10^{-3} (0.2%) for fasted adults and 2×10^{-4} (0.02%) for fed adults. Additional studies are needed to determine the relevance of GI absorption values in fasted animals to the establishment of drinking water standards. The GI absorption of plutonium complexed to humic acids in drinking water should also be considered. Studies in these areas are currently under way in our laboratory.

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